The relation between protein intake and body composition in obese older adults with diabetes type 2.
Acknowledgements

In this thesis you will read about our study on the relation between protein intake and body composition in older adults with obesity and diabetes type 2. The thesis was written for the Bachelor Nutrition and Dietetics at the Amsterdam University of Applied Sciences. The thesis was written in a period of 20 weeks and was commissioned by Dr. P.J.M. Weijs. The data used for this thesis were obtained from the baseline data of five cohorts of the PROBE study.

The base for our research question was suggested by Robert Memelink, coordinator of the PROBE study. Both he and our mentor Amely Verreijen were of great help and supported us throughout the thesis period.

We would like to thank the following people for their support during the thesis period. First of all, we would like to thank our mentor, Amely Verreijen. Not only was she of great support in writing our thesis, she also learned us how to interpreted statistical analysis. This helped us to reach the fullest potential of our thesis. Furthermore, we would like to thank Robert Memelink for giving us a clear start at the beginning of our thesis period. The support of Robert and Amely helped us to complete all five databases of the PROBE study. With these complete datasets we could explore all data to the fullest for this thesis.

We wish you a pleasant reading.

Noach Meged and Lisette Schreurs
Abstract

Introduction: The current daily recommendation of dietary protein for adults is 0.8 g · kg BW$^{-1}$ · d$^{-1}$. Age-related loss of fat-free mass is partly the result of insufficient protein intake in older adults. Recent studies show that a daily intake of 1.2 – 1.5g · kg BW$^{-1}$ of dietary protein may be needed to preserve the muscle tissue in older adults who suffer from acute or chronic diseases. So far, only few studies focused on protein intake and body composition in older adult with obesity and diabetes type 2. Because of reduced insulin functioning, a possible relation between protein intake and fat-free mass can be different in diabetic older adults compared to non-diabetic older adults. The objective of this study was to investigate whether there is a relation between protein intake and body composition in older adults with obesity and diabetes type 2 aged 55-85.

Methods: This study included 122 older adults with diabetes type 2 and obesity. PROBE baseline data was used for data analysis. Parameters for body composition were appendicular lean mass, fat free mass and fat mass. Protein intake (g · kg BW$^{-1}$ · d$^{-1}$) was based on 3-day food records. Body composition was measured by dual-energy X-ray absorptiometry. ANOVA compared for significant differences between the protein categorisations (≤0.8 g · kg adj BW$^{-1}$ · d$^{-1}$, 0.8 – 1.2 g · kg adj BW$^{-1}$ · d$^{-1}$ and ≥1.2 g · kg adj BW$^{-1}$ · d$^{-1}$). Adjusted weight was implemented in the figures to correct for body weight. Regression analysis is executed with body composition as outcome variables and protein intake as main determinant. Body weight was included as confounder.

Results: The mean age of the subjects is 66.6 ± 5.0 years, 64.5% men, 35.5% female. Mean protein intake is 1.0 ± 0.34 g · kg adj BW$^{-1}$ · d$^{-1}$. In the study group 9 (20%) of the participating women (n=43) and 22 (28%) of the men (n=78) met the protein recommendations of 1.2 g · kg BW$^{-1}$ · d$^{-1}$ or higher. 1 g · kg BW$^{-1}$ · d$^{-1}$ extra protein associated positive with ALM (bèta is 3.1 kg ± 1.1 SE, P=0.006, 95% CI: 0.9 ; 5.3 kg). 1 g · kg BW$^{-1}$ · d$^{-1}$ extra protein associated positive with FFM (bèta is 6.3 kg ± 2.3 SE, P=0.008, 95% CI: 1.6 ; 10.9). 1 g · kg BW$^{-1}$ · d$^{-1}$ extra protein associated negative with FM (bèta is -6.5 kg ± 2.1 SE, P= 0.002, 95% CI: -10.67 ; -2.351).

Conclusion: Adequate protein intake relates positive with appendicular lean mass and fat free mass, while fat mass shows a negative relation. These findings support evidence for a higher protein intake for older adults with obesity and type 2 diabetes to preserve FFM. This study acknowledges protein intake as one of the many factors that influences muscle protein synthesis. Explorative cross-sectional study can’t conclude a causality. Results should always be supported by other (future) studies.

Key-words: Appendicular lean mass, fat-free mass, fat mass, body composition, obese older adults with diabetes type 2, cross-sectional study, protein intake
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1. Introduction

The prevalence of adults aged 65 years and older has increased the past decade. It is expected that 26% of the population will be 65 years and older in 2040 (1). That is a relevant difference compared to the elderly population in the year 2012, where the percentage of elderly inhabitants was only 16% (1). One of the reasons of an increase in older adult is a longer life expectancy. The improvement of the medical and technological sectors under influence of scientific research in the past 60 years, lowered the risk of death from heart and cardiac diseases drastically (2).

Physical functioning declines when a person becomes older (3). Physical activity is necessary to stimulate muscle synthesis. Inactivity can lead to reduced muscle synthesis, increasing the risk of fat-free mass (FFM) loss (8). In general, muscle tissue starts to decrease around the 4th decade. The fat mass (FM) percentage increases from 10-30% during aging, until its stabilizes around 40-45% at the age of 60-65 (3,4). The total amount of fat in older adults is redistributed in the abdominal area of the body. At the same time the FFM is decreasing with 40%, especially the muscle tissue. The combination of increased fat mass and decreasing FFM can lead to a medical condition known as sarcopenic obesity (3,4). The decrease of lean body mass and decline of physical performance leads to negative health outcomes such as disability, higher morbidity and mortality (5,6). Preservation of FFM is an essential factor to maintain the quality of life (7,8). Meta-analysis reveals a 24% increased risk of all-cause mortality in sarcopenic obese patients (9). Several cross-sectional studies associate low FFM with higher cardiovascular risk profiles including hyperglycaemia, hypertension, insulin resistance, dyslipidaemia, and lower cardiorespiratory fitness (10-12).

Alongside physical activity, dietary protein can stimulate an anabolic environment to maintain muscle tissue (13,14). The current daily recommendation of dietary protein for adults is 0.8 g · kg BW^{-1} · d^{-1} (20). Nutritional surveys suggest age-related loss of FFM is partly the result of insufficient protein intake by older adults (6,15). Recent studies show that a daily intake of 1.2 – 1.5g · kg BW^{-1} of dietary protein may be needed to preserve the muscle tissue of older adults who suffer from acute or chronic diseases (16-18). Houston et al. (15) demonstrated that older adults with a daily protein intake of 1.2 ± 0.4 g · kg BW^{-1} · d^{-1} lost 40% less FFM and appendicular lean mass (ALM) compared to older adults with a daily protein intake of 0.8 ± 0.3 g · kg BW^{-1} · d^{-1}, over a 3 year period (15).

To our knowledge only a couple of studies investigated the relation between protein intake and body composition. Houston et al. (15) indicates the influence of dietary protein in older adults as described above. Sahni et al. (19) concludes cross sectional, that a protein intake of at least 1.2g · kg BW^{-1} · d^{-1} may be beneficial for the preservation of FFM and quadriceps muscle strength in older adults. McDonald et al. (26) reveals the maintenance of lean body mass in older adults with a protein intake of 1.26 g · kg BW^{-1} · day^{-1} in a 6 year period. However, so far only few studies focused on protein intake and body composition in older adults with obesity and diabetes type 2. Characteristics of type 2 diabetes is the loss of endocrine and exocrine functions in the pancreas. Incretins secretion and insulin function is decreased in type 2 diabetes subjects (21). Incretins play an important role in the postprandial blood glucose regulation by stimulating insulin secretion and saturating individuals appetite. A study of Jakubowicz et al. (22) showed that a high protein snack before breakfast promotes the activity of incretins in diabetic patients, which resulted in beneficial postprandial blood glucose levels due to an increased insulin secretion. Insulin is known as an anabolic hormone, vital for proteins/amino acids digestion and prevention of FFM breakdown (25). Because of this reduced insulin functioning, a possible relation between protein intake and FFM can be different in diabetic older adults compared to non-diabetic older adults. Therefore the objective of this study is to investigate whether there is a relation between protein intake and body composition in older adults with obesity and diabetes type 2. To our knowledge so far no studies are focussed on the association between protein intake and muscle mass and body composition in a population of older obese adults.
with type 2 diabetes. Based on previous studies, we hypothesize that a higher amount of dietary protein intake will associate in a higher number of appendicular lean mass.
2. Methods

2.1 Subjects

This study included 122 older adults aged 55 – 85 years old with diabetes type 2 and obesity. The inclusion criteria were older adults between the age of 55 and 85 years old, diabetes mellitus type 2 with the use of related medication or a HbA1c ≥43 mmol/mol, a BMI ≥30.0 kg/m² or a BMI of 27.0 kg/m² in combination with a waist circumference >88 cm for women and >102 cm for men. Subjects were excluded in case of alcohol or drug abuse, involuntary weight loss, hepatic diseases, renal disease (eGFR <60 ml/min), chest pains, myocardial infarctions and/or cardiac surgery within 3 months prior to the baseline visit, any malignant disease during the last five years except for the adequately treated prostate cancer without evidence of metastases, localised bladder cancer, cervical carcinoma in situ, breast cancer in situ, non-melanoma skin cancer, and other relevant medical history that could affect the study outcome.

The participants were recruited by advertising in a local newspaper and spreading brochures via general practitioners. A written informed consent was obtained from all subjects. An informed consent had to be signed before participating in the PROBE-study (described below, paragraph 2.2). The PROBE-study was approved by the Medical Review and Ethics Committee of the Foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO) in Assen (2014/17264). The study is registered at the Dutch Trial Register (NTR4497; http://trialregister.nl).

2.2 Study design

Cross-sectional analysis was performed to analyse the relation between protein intake and body composition in obese older adults with diabetes type 2, using baseline data of the PROBE-study. Figure 1 displays the design of this cross sectional study. PROBE is a randomized double-blind intervention that studies the impact of a high whey protein-, leucine-, and vitamin D-enriched supplement on the preservation of muscle tissue during a weight loss intervention in obese older adults with diabetes type 2. The included participants followed a 13 week weight loss program. This program consisted of a hypocaloric diet under the supervision of a dietician and an exercise program 3 times a week. For this thesis only baseline data were used to answer the research question.

2.2.1 Dietary intake

The protein intake in grams per kilogram body weight per day was estimated by a calculation of the nutrients in the 3-day food record. The 3-day food record is a three day notation of the nutritional intake filled out by the study participants. Two workdays and one weekend day were written down to simulate the weekly intake pattern. Each day contained a format with seven possible mealtimes. Participants reported product type and amount for each mealtime. Nutrients, including protein in grams, were calculated by using the Nevo-Tabel 2013 (28). The 3-day food records were checked and eventually included, if they met the minimum notation of 2 days. Incomplete 3-day food records and 3-day physical activity records were excluded from this study to ensure good quality records. Mean protein intake was calculated as total protein intake divided by three days. Protein intake (g · kg BW⁻¹ · d⁻¹) was calculated as average protein intake per day divided by body weight (BW) in kg. Total protein g · kg BW⁻¹ · d⁻¹ is the preferred index for the analyses, making the results comparable with other studies. Protein g · kg BW⁻¹ · d⁻¹ is also considered valuable in practical use. Dieticians determine protein needs based on protein g · kg BW⁻¹ · d⁻¹ (35).
2.2.2 Body composition

Body composition was measured at baseline. Body weight was measured in kilograms by using a scale of an air displacement plethysmography (Bodpod, Life Measurement Inc., Concord (CA), USA). Previous to the measurement, the scale of the Bodpod was calibrated using two 10 kg weights. Subjects were asked to remove all jewellery, piercings and hairpins and to only wear underwear during measurements. To reliably measure body weight, the subjects had to fast ≥6 hours prior to baseline. Hence, the measurements took place early in the morning. Height was measured in centimeters by using a digital wall-mounted stadiometer (De Grood productions, LLM 250D, serial number 270901).

Body composition data was acquired by the Hologic Discovery dual-energy X-ray absorptiometry (Trump Medical BV), also called DXA. A whole body scan was used to obtain body composition data. Quality was kept by the daily radiographic uniformity quality control. During the DXA scans the subjects feet were locked into place to expose all bone structures to the scan. This way the bone mineral content (BMC) and the bone mineral density (BMD) were calculated correctly by the densitometer. Duplicates of the right arm mass replaced left arm mass if the subjects left arm did not fit completely into the DXA scan surface. Hologic APEX Software version 4.0.2 was used to calculate body composition. The sum of the body tissue scans corresponded with the body weight in kg, measured on the BODPOD daily calibrated scale during baseline. The DXA scan was able to partition the body tissue into three fractions: bone mineral content (BMC), fat mass (FM) and lean mass total (LM). Appendicular lean mass (ALM) was defined and used as a proxy for muscle mass. Studies with older adults often use ALM as a measure of muscle mass (15). ALM was defined as the sum of lean mass (without bone) of both arms and legs. Fat-free mass (FFM) was acquired by the sum of all LM and BMC. The total amount of FM in kg was calculated as the sum of all fat mass (kg) of legs, arms, trunk and head.

![Diagram](FIGURE 1: Study design cross-sectional research)
2.2.3 Potential confounders

The variables energy intake, age, sex, race, physical activity, smoking, alcohol consumption and duration of diabetes mellitus type 2 were potential confounders and previously defined as confounder in the relation between protein intake and change in lean mass (15). Demographic characteristics (i.e., age, sex, race), smoking, alcohol consumption, duration of diabetes mellitus type 2 and comorbidity were enquired during screening. Level of physical activity was estimated by a 3-day physical activity record. All included subjects filled in a 3-day physical activity record which were checked for completeness during the baseline visit. Smoking and alcohol consumption were categorized as yes or no.

2.3 Data analysis

Double-data entry was used and inconsistencies were checked and solved. Analyses of the composition and protein intake data were done by IBM SPSS Statistics software for Windows, version 22. Protein intake (g · kg adj BW⁻¹ · d⁻¹) was implemented in the figures to correct for body weight. Subjects with a BMI ≥30.0 kg/m² body weight were adjusted by calculating the body weight at BMI 27.5 kg/m² (29,30). Categorisation of the protein intake in g · kg adj BW⁻¹ · d⁻¹ was chosen to visualize and compare the intake amounts (≤0.8 g · kg adj BW⁻¹ · d⁻¹, 0.8 – 1.2 g · kg adj BW⁻¹ · d⁻¹ and ≥1.2 g · kg adj BW⁻¹ · d⁻¹). ANOVA compared for significant differences between the protein categorisations. Linear regression analysed the relation between the dependent and independent variables. The dependent variables were fat mass (kg), fat-free mass (kg) and the appendicular lean mass (kg). Regression models were used to correct for confounding variables in order to estimate the association to reduce bias. Protein intake (g · kg BW⁻¹ · d⁻¹) is the independent variable in the regression models. Confounding variables were added one by one to the crude regression model, checking whether the beta for protein intake (β₁) changed. Starting with the variable body weight that was considered to have the most effect on the beta one for protein. The beta for protein was interpreted as the change in the mean of Y-axis for a one-unit increase in protein intake (x-axis) (31). A variable was assumed to be a confounding variable if it effected the beta for protein by more than 10%. A P value of < 0.05 was considered statistically significant.
3. Results

3.1 Subjects and level of compliance

In total 122 subjects of the PROBE-study were included in this cross-sectional analysis. Characteristics of the subjects are presented in Table 1. Of the subjects 65.5% was male and 35.5% was female. All subjects were included in the period September 2014 until November 2016. At the time of the baseline, average age was 66.6 ± 5.0 years (mean ± SD).

| TABLE 1 Baseline characteristics of subjects from the PROBE-study1 |
|-----------------|-----------------|-----------------|-----------------|
|                 | Men             | Women           | Total           |
| Subjects, n (%) | 78 (64.5%)      | 43 (35.5%)      | 122 (100%)      |
| Age, y          | 66.8 ± 6.1      | 66.0 ± 5.8      | 66.6 ± 5.0      |
| Height, cm      | 177.5 ± 7.0     | 165.1 ± 6.1     | 173.1 ± 8.9     |
| Body weight (BW), kg | 102.5 ± 14.0 | 92.9 ± 15.9 | 99.1 ± 15.3 |
| BMI, kg/m²      | 32.5 ± 3.6      | 34.1 ± 5.6      | 33.0 ± 4.5      |
| Total protein intake, g/d | 92.0 ± 32.2 | 73.8 ± 20.2 | 85.2 ± 29.6 |
| Appendicular lean mass, kg | 29.9 ± 3.9 | 22.0 ± 2.9 | 27.1 ± 5.2 |
| Fat mass, kg    | 32.0 ± 7.7      | 38.8 ± 10.4     | 34.4 ± 9.2      |
| Fat free mass, kg | 72.2 ± 8.8      | 56.0 ± 7.1      | 66.5 ± 11.3     |

1 Values are means ± SDs or percentage %

3.2 Protein intake

The mean protein intake of the complete study group is 85.2 ± 29.6 g/d (mean ± SD), which corresponds with 0.87 ± 0.29 g · kg BW⁻¹ · d⁻¹. Men show a mean protein intake of 92.0 ± 32.2 g/d, women show a mean protein intake of 73.8 g/d ± 20.2. In the study group 9 (20%) of the participating women (n=43) and 22 (29%) of the men (n=78) met the protein recommendations of 1.2 g · kg BW⁻¹ · d⁻¹ or higher (15). The total study population shows a mean protein intake of 1.0 ± 0.34 g · kg adj BW⁻¹ · d⁻¹ when corrected for body weight.

3.3 Body composition

3.3.1 Appendicular lean mass

Figure 1 displays the association between ALM (kg) and protein intake (g · kg adj BW⁻¹ · d⁻¹) categorized in three categories according to protein intake (≤0.8 g · kg adj BW⁻¹ · d⁻¹, 0.8 – 1.2 g · kg adj BW⁻¹ · d⁻¹ and ≥1.2 g · kg adj BW⁻¹ · d⁻¹). Results are presented for men (n=78) and women (n=43) separately. No statistical significant differences in ALM were found between the three groups of protein intake (P = 0.59).
The association between protein intake in g per kg adjusted body weight and appendicular lean mass in men and women. Values are means and error bars represent the SD.

**TABLE 2**: The association between appendicular lean mass (kg) and protein intake (g · kg BW⁻¹ · d⁻¹) in the PROBE study subjects.

<table>
<thead>
<tr>
<th>Protein intake (g/kg BW)</th>
<th>β₁ (SE)</th>
<th>P value</th>
<th>95% CI</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1¹</td>
<td>0.16 (1.67)</td>
<td>0.92</td>
<td>-3.14, 3.46</td>
<td>0.00</td>
</tr>
<tr>
<td>Model 2²</td>
<td>3.13 (1.12)</td>
<td>0.01</td>
<td>0.92, 5.34</td>
<td>0.58</td>
</tr>
</tbody>
</table>

¹ Model 1 is the crude model without any corrections.
² Model 2 is corrected for determinant body weight.

The association between appendicular lean mass (kg) and protein intake (g · kg BW⁻¹ · d⁻¹) in the PROBE study subjects is presented in Table 2. Regression analysis showed a positive significant association between protein and appendicular lean mass with β 3.1 kg ± SD 1.1 and 95% CI 0.92; 5.3. In other words, 1 gram of extra protein per kilogram body weight per day is associated with 3.1 kg more appendicular lean mass (P = 0.01). Other potential confounders taken into consideration did not changed β with more than 10% in the regression analysis.

### 3.3.2 Fat-free mass

Results are presented for men (n=78) and women (n=43) separately. ANOVA demonstrates no significant similarities (P = 0.63) between the categorized groups. **Figure 2** displays the association between fat-free mass (kg) and protein intake (g · kg adj BW⁻¹ · d⁻¹) categorized in three categories according to protein intake.
FIGURE 2: Association between protein intake in g per kg adjusted body weight and fat-free mass in women and men. Values are means and error bars represent the SD.

TABLE 3: Association between free-fat mass (kg) and protein intake (g · kg adj BW\(^{-1}\) · d\(^{-1}\)) in the PROBE study subjects.

<table>
<thead>
<tr>
<th>Total protein intake g/kg BW</th>
<th>( \beta_1 ) (SED)</th>
<th>P waarde</th>
<th>95% CI</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1(^1)</td>
<td>-36 (3.63)</td>
<td>0.92</td>
<td>-7.56, 6.84</td>
<td>0.00</td>
</tr>
<tr>
<td>Model 2(^2)</td>
<td>6.29 (2.35)</td>
<td>0.01</td>
<td>1.64, 10.94</td>
<td>0.61</td>
</tr>
</tbody>
</table>

\(^1\) Model 1 is the crude model without any corrections.

\(^2\) Model 2 is corrected for determinant body weight.

The association between fat-free mass (kg) and protein intake (g · kg adj BW\(^{-1}\) · d\(^{-1}\)) in PROBE study subjects is presented in Table 3. Regression analysis showed a positive significant association between protein intake and fat-free mass. 1 gram extra protein per kilogram body weight per day is associated with 6.3 kg more fat-free mass (P = 0.008). Other potential confounders taken into consideration did not change \( \beta \) with more than 10% in the regression analysis.

3.3.3 Fat mass

Results are presented for men (n=78) and women (n=43) separately. ANOVA demonstrates no significant similarities (P = 0.81) between the categorized groups. Figure 3 displays the association between fat mass (kg) and protein intake (g · kg adj BW\(^{-1}\) · d\(^{-1}\)) categorized in three categories according to protein intake.
FIGURE 3: Association between protein intake in g per kg adjusted body weight and fat mass in woman and men. Values are means and error bars represent the SD.

TABLE 4: Association between fat mass (kg) and protein intake (g · kg BW⁻¹ · d⁻¹) in the PROBE study subjects.

<table>
<thead>
<tr>
<th>Fat mass</th>
<th>Total protein intake g/kg BW</th>
<th>β₁ (SD)</th>
<th>P waarde</th>
<th>95% CI</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.8</td>
<td>Model 1¹</td>
<td>-10.908 (2.770)</td>
<td>&lt;0.001</td>
<td>-16.394, -5.422</td>
<td>0.119</td>
</tr>
<tr>
<td>0.8 - 1.2</td>
<td>Model 2²</td>
<td>-6.511 (2.100)</td>
<td>0.002</td>
<td>-10.671, -2.351</td>
<td>0.521</td>
</tr>
<tr>
<td>≥1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Model 1 is the crude model without any corrections.
² Model 2 is corrected for determinant body weight.

The association between fat mass (kg) and protein intake (g · kg BW⁻¹ · d⁻¹) in the study subjects is presented in Table 4. Regression analysis showed a negative significant association between protein intake and fat mass with $β_1 \cdot -6.5 ± SD 2.1$ and 95% CI (-10.67, -2.351). In other words, 1 gram extra protein per kilogram body weight per day is associated with 6.5 kg less fat mass ($P = 0.002$). Other potential confounders taken into consideration weren’t significant in the regression analysis.
4. Discussion

This cross-sectional study is the first to explore the relation between protein intake and body composition in older adults with obesity and diabetes type 2. This study reveals a positive relation between protein intake $g \cdot kg\ BW^{-1} \cdot d^{-1}$ and the appendicular lean mass in both men and women, supporting evidence for a higher protein for older adults intake. A higher total protein in grams per day was hypothesized to result in a higher appendicular lean mass in older adults with obesity and diabetes type 2 a positive association was affirmed by the results in this study. The relation between protein intake and body composition contributes to the understanding in the development of sarcopenia. This is an explorative cross-sectional study, therefore no causality can be made. The results should always be supported by other (future) studies. The set of results can provide evidence to adjust current dietary guidelines for older adults with obesity and diabetes type 2. The optimal protein intake for older adults is still open to discussion due to varying results and recommendations to prevent sarcopenia (3,8-19). The current recommendation for protein intake is $0.8 \ g \cdot kg^{-1} \ body \ weight \ for \ adults$, including older adults (33), while recent studies reveal evidence for a higher protein intake. Houston et al. (15) reported that older adults with a mean protein intake of $1.2 \pm 0.4 \ g \cdot kg\ BW^{-1} \cdot d^{-1}$ lost 40% less appendicular lean mass compared with subjects with a mean protein intake of $0.8 \pm 0.3 \ g \cdot kg\ BW^{-1} \cdot d^{-1}$ in a 3 year period. McDonald et al. (26) reveals the maintenance of lean body mass in older adults with a protein intake of $1.26 \ g \cdot kg\ BW^{-1} \cdot day^{-1}$ in a 6 year period. Previous study show that a daily intake of $1.2-1.5 \ g \· kg\ BW^{-1} \cdot d^{-1}$ of dietary protein may be needed to preserve the muscle tissue in older adults who suffer from acute or chronic diseases (34). Characteristics of type 2 diabetes is the loss of endocrine and exocrine functions in the pancreas. The decreased secretion of insulin in type 2 diabetes elders implies a disordered protein/amino acids digestion (21,22). Nevertheless older adults can be able to stimulate muscle protein synthesis with an adequate protein intake (15,26,32).

The protein intake is just one of the many factors that influences muscle protein synthesis and breakdown. Witard et al. (27) presents an overview of other factors that influences the regulation of skeletal muscle mass, see appendix II. Apart from the total protein intake, the amount of protein per meal seem to have an impact in muscle preservation. Studies confirm a minimum intake of 25 g high-quality protein per meal is required to stimulate muscle protein synthesises (22,24,36,37). Yet, this study did not investigate possible -effects of protein intake per meal on body composition in this study group. Future analysis of the protein intake per meal in obese older adults with diabetes type 2 can explore the impact on muscle preservation. Protein type is another factor that might influence body composition. However, this factor isn’t taken into account in this thesis. Evidence suggest that protein source and composition plays a role in the promotion of muscle mass synthesis (1,2,15). It has been shown that dairy protein is more effective in stimulating muscle protein mass in comparison with plant protein. When looking at older adults, 30 g of whey protein or more would stimulate an even higher muscle protein synthesis (38,39). Further research of the protein intake is needed to clarify the impact between animal and plant protein in the PROBE subjects.

Protein needs is currently established on nitrogen balance data. Although measurements in protein requirements are often established using nitrogen balance data, the validity of this method is currently under debate amongst researchers. Zero difference in nitrogen intake and nitrogen loss are considered the most interesting and valuable. Nevertheless for unknown reason positive nitrogen errors occur systematically in nitrogen balance analysis (49). There is no consensus on how this positive error should be interpreted. A study confirms that high nitrogen balances shouldn’t be observed as biological real, making the current method questionable (50). Additional research is needed to find solutions for the errors in the nitrogen measurement method.
The regression analysis in this study showed a negative association between protein intake and fat mass. Current scientific studies show similar results. Clinical trials measured higher amounts of fat mass loss in high protein diets (1.07–1.60 g · kg BW \(^{-1} \cdot \text{d}^{-1}\)) compared with low protein diets (0.55–0.88 g · kg BW \(^{-1} \cdot \text{d}^{-1}\)) (51). There is still disagreement about the correct fat-loss strategy. Soenen et al. supports that energy restriction and 0.8 g · kg BW \(^{-1} \cdot \text{d}^{-1}\) of protein is sufficient enough for fat loss, while 1.2 g is fundamental for the preservation of FFM (52). Meta-analysis shows 1.2 g · kg BW \(^{-1} \cdot \text{d}^{-1}\) of dietary protein intake can improve weight managements in short term. Long term results were not beneficial due to the lack of compliance in the subjects (53).

One of the strengths of this study is the use of a valid instrument to measure body composition. Dual-energy X-ray absorptiometry whole body scans provide accurate analysis of particular body parts (40). Apart from handgrip strength and gait speed, the DXA is broadly used to define sarcopenia in older adults by the amount of appendicular lean mass (41,42). Studies with the DXA can’t always be strictly compared. Past results showed significant differences between Hologic and GE Lunar DXA scans (43). Lean tissue like skin, blood and connective tissue cannot be distinguished by the DXA making the measurements less accurate in comparison with MRI or CT scans, which are considered to be the golden standard (44). The association between subcutaneous adiposity and total fat mass diminishes during ageing, making body composition measurements less accurate in older adults (45). Therefore previous studies strongly suggest a set of reference values to standardize the body composition assessment (46). Strict standardization of the body composition assessment was carried out in the PROBE study to minimize irregularities. Incomplete 3-day food records and 3-day physical activity records were excluded from this study to ensure good quality records. An inaccurate energy report could have influenced the total protein intake. Energy intake in the 3-day food records are known to be inaccurate by earlier reports (46). Individual dietary counseling could have improved the energy intake estimations (47,48). Although the nature of this cross-sectional study makes energy needs difficult to assess.

In conclusion, protein intake relates positively with appendicular lean mass and fat-free mass, while fat mass shows a negative relation. These findings support evidence for a higher protein intake for older adults with obesity and type 2 diabetes to preserve FFM. It’s plausible that a sufficient quantities of dietary protein can reduce the risk of sarcopenia in older adults during a weight loss program. Based on the results, we can generally recommend dieticians to take an adequate protein intake into consideration to reduce the loss of fat-free mass during individual dietary counseling. Preservation of fat-free mass is an essential factor to maintain physical performance and quality of life. A sufficient number of fat-free mass lowers the risks of disability, morbidity and mortality. However, protein dose is just one of the many factors in the process of preserving lean mass. This explorative cross-sectional study can’t conclude a causality. Results should always be supported by other (future) studies.
References


Appendix I: Syntax

TABLE 1: Characteristics subjects
DATASET ACTIVATE DataSet1.
DESCRIPTIVES VARIABLES=SEX BIRTHDATE Height BPWEIGHT BMI EIWIT ALM_DXAkg FFM_DXAkg DXA_FMkg
/STATISTICS=MEAN STDDEV MIN MAX.
SORT CASES BY SEX.
SPLIT FILE LAYERED BY SEX.
DESCRIPTIVES VARIABLES=SEX BIRTHDATE Height BPWEIGHT BMI EIWIT ALM_DXAkg FFM_DXAkg DXA_FMkg
/STATISTICS=MEAN STDDEV MIN MAX.
SPLIT FILE OFF.

ADJUST BW PROTEIN G/KG
RECODE BMI (30 thru Highest=Copy) INTO BMIAjusted.
COMPUTE ADJUSTEDBW=BPWEIGHT * 27.5 / BMI.
COMPUTE EIWITgKGaw=EIWIT / ADJUSTEDBW.
RECODE EIWITgKGdplusADW (0 thru 0.79999999999999999=1) (0.8 thru 1.19999999=2) (1.2 thru Highest=3) INTO EIWITcatAW.

COMPARE MEANS ANOVA
ONEWAY ALM_DXAkg BY EIWITcatAW
/MISSING ANALYSIS.
ONEWAY FFM_DXAkg BY EIWITcatAW
/MISSING ANALYSIS.
ONEWAY DXA_FMkg BY EIWITcatAW
/MISSING ANALYSIS.

FIGURE 2: association ALM and protein
SORT CASES BY SEX.
SPLIT FILE LAYERED BY SEX.
MEANS TABLES=ALM_DXAkg BY EIWITcatAW
/CELLS=MEAN COUNT STDDEV.

TABLE 2: association ALM and protein
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT ALM_DXAkg
/METHOD=ENTER EIWITgKGd.

REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT ALM_DXAkg
/METHOD=ENTER EIWITgKGd BPWEIGHT.

TABLE 3: association FFM and protein
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT DXA_FMkg
/METHOD=ENTER EIWITgKGd.

REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT DXA_FMkg
/METHOD=ENTER EIWITgKGd BPWEIGHT.

FIGURE 3: association FFM and protein
SORT CASES BY SEX.
SPLIT FILE LAYERED BY SEX.
MEANS TABLES=FFM_DXAkg BY EIWITcatAW
/CELLS=MEAN COUNT STDDEV.

TABLE 4: association FM and protein
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT FFM_DXAkg
/METHOD=ENTER EIWITgKGd.
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGIN
/DEPENDENT FFM_DXAkg
/METHOD=ENTER EIWITgKg BPWEIGHT.

Figure 4: association FM and protein
SORT CASES BY SEX.
SPLIT FILE LAYERED BY SEX.
MEANS TABLES=FM_DXAkg BY EIWITcatAW
/CELLS=MEAN COUNT STDDEV.
Appendix II: Witard et al. figure 4

Figure 5: Simplified diagram detailing the role of amino acid availability in regulating muscle protein synthesis with amino acid/protein ingestion and exercise. Whilst resistance exercise preferentially stimulates the synthesis of contractile myofibrillar proteins (e.g., actin, myosin, troponin), resistance exercise also stimulates the synthesis of non-contractile proteins (e.g., mitochondrial and sarcoplasmic) in skeletal muscle.
# Appendix III: Beoordelingsformulier: scriptie afstudeerproject (AP) Voeding & Diëtetiek, Hogeschool van Amsterdam

<table>
<thead>
<tr>
<th>Naam student:</th>
<th>Noach Meged (500623156) en Lisette Schreurs (500697345)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titel en nummer AO</td>
<td>The relation between protein intake and body composition in obese older adults with diabetes type 2. (AO nummer: 2017100)</td>
</tr>
<tr>
<td>Opdrachtgever</td>
<td>Peter Weijs</td>
</tr>
<tr>
<td>Naam examinator:</td>
<td>Anouk van der Steen</td>
</tr>
<tr>
<td>Naam docentbegeleider:</td>
<td>Amely Verreijen</td>
</tr>
<tr>
<td>Datum:</td>
<td>8 januari 2017</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Criterium</th>
<th>B</th>
<th>H</th>
<th>Onvoldoende</th>
<th>voldoende</th>
<th>goed</th>
<th>Score o/v/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randvoorwaardelijke eisen (weging 0%)</td>
<td>3</td>
<td>I</td>
<td><strong>Niet op tijd ingeleverd Leesbaarheid</strong></td>
<td>De afstudeeropdracht is een aaneenschakeling van stukken tekst, het geheel loopt niet. Een eigen schrijfstijl is niet herkenbaar. Er zijn veel grammaticale en spellfouten. De formulering is persoonlijk, subjectief, vaag en wijdlopig. <strong>Structuur</strong></td>
<td>De indeling in hoofdstukken, paragrafen en alinea’s is niet duidelijk van de probleemstelling afgeleid. De titel van de afstudeeropdracht dekt de lading niet en/of de kopjes van de paragrafen dekken de inhoud van de paragraaf niet. <strong>Lay-out</strong></td>
<td>De lay-out is rommelig. Beelden, tabellen en figuren ondersteunen de tekst en argumenten niet. <strong>Omvang</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Op tijd ingeleverd Leesbaarheid</td>
<td>De afstudeeropdracht leest redelijk vlot, er zijn weinig grammaticale en spellfouten. De formulering is bondig, hier en daar wat algemeen en niet overal even formeel. Het verhaal is een lopend geheel. <strong>Structuur</strong></td>
<td>De hoofdstukindeling is logisch afgeleid van de probleemstelling, maar de onderverdeling op lager niveau is voor verbetering vatbaar. De titels van de afstudeeropdracht, de hoofdstukken en paragrafen zijn niet altijd kernachtig geformuleerd en representatief voor de inhoud. <strong>Lay-out</strong></td>
<td>De lay-out is functioneel en professioneel. Gebruik van beelden, tabellen en figuren is functioneel en bijlagen worden benut waar nodig. <strong>Omvang</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Op tijd ingeleverd Leesbaarheid</td>
<td>De afstudeeropdracht is vlot leesbaar en in correct Nederlands of Engels geschreven: de formulering is bondig, concreet en formeel. De rode draad in het verhaal is duidelijk. <strong>Structuur</strong></td>
<td>De indeling in hoofdstukken, paragrafen en alinea’s is logisch afgeleid van de probleemstelling en duidelijk De titels van de afstudeeropdracht, de hoofdstukken en paragrafen zijn kernachtig geformuleerd en representatief voor het geheel. Ze kondigen de inhoud correct aan. <strong>Lay-out</strong></td>
<td>De lay-out is functioneel en professioneel. Gebruik van beelden, tabellen en figuren is functioneel en bijlagen worden benut waar nodig. <strong>Omvang</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Criterium</th>
<th>B</th>
<th>H</th>
<th>Cijfer 1,0-5,0</th>
<th>Cijfer 5,5-7,5</th>
<th>Cijfer 8-10</th>
<th>Cijfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. correcte definitie en consistente uitwerking probleemstelling</td>
<td>1</td>
<td>3</td>
<td>I II</td>
<td>Als niet aan de criteria voor 5,5-7,5 is voldaan.</td>
<td>De probleemstelling (en deelvragen) worden systematisch uitgewerkt in 55-75% van de scriptie</td>
<td>De probleemstelling (en deelvragen) worden systematisch uitgewerkt in 80-100% van de scriptie.</td>
</tr>
<tr>
<td>Toelichting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. verantwoording (onderzoeks)methode</td>
<td></td>
<td></td>
<td></td>
<td>Als niet aan de criteria voor 5,5-7,5 is voldaan.</td>
<td>De gekozen methode sluit aan bij de probleemstelling en wordt inzichtelijk beargumenteerd. (De methode staat zodanig beschreven dat het onderzoek herhaalbaar is)</td>
<td>+ De gekozen methode is informatiever beschreven en wetenschappelijk onderbouwd.</td>
</tr>
<tr>
<td>Toelichting</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. juiste weergave resultaten</td>
<td></td>
<td></td>
<td></td>
<td>Als niet aan de criteria voor 5,5-7,5 is voldaan.</td>
<td>De analyse van gegevens is correct uitgevoerd De resultaten staan objectief, correct en overzichtelijk beschreven/gepresenteerd (resultaten komen voort uit het onderzoek). Tabellen en figuren zijn conform regels.</td>
<td>+ De belangrijkste resultaten worden gescheiden van de minder belangrijke (bv in bijlage) in relatie tot de probleemstelling.</td>
</tr>
<tr>
<td>Toelichting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. juistheid, opbouw en niveau van discussie en conclusie</td>
<td></td>
<td></td>
<td></td>
<td>Als niet aan de criteria voor 5,5-7,5 is voldaan.</td>
<td>De resultaten worden correct geïnterpreteerd en vergeleken met de literatuur Conclusie geeft antwoord op de probleemstelling. Sterke en zwakke punt worden correct benoemd.</td>
<td>+ De helicopterview wordt toegepast, waarbij relevante ontwikkelingen in de beroepsoefening en aanverwante domeinen zijn beschreven.</td>
</tr>
<tr>
<td>Toelichting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. concrete aanbevelingen die voortkomen uit het</td>
<td></td>
<td></td>
<td></td>
<td>Als niet aan de criteria voor 5,5-7,5 is voldaan.</td>
<td>Aanbevelingen vloeien logisch voort uit de discussie en conclusie.</td>
<td>+ De aanbevelingen zijn bruikbaar voor de organisatie en voor het gehele werkveld.</td>
</tr>
</tbody>
</table>
onderzoek/opdracht
(indien eindproduct in bijlage zit wel meerekenen)  
+ De aanbevelingen zijn creatief en innovatief  
+ Er wordt een transfer gemaakt naar (ontwikkelingen in) andere werkvelden.

<table>
<thead>
<tr>
<th>Criterium</th>
<th>B</th>
<th>H</th>
<th>Cijfer 1-5,5</th>
<th>Cijfer 5,5-7,5</th>
<th>Cijfer 8-10</th>
<th>Cijfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. niveau en actualiteit (Engelstalige) literatuur</td>
<td>7</td>
<td>I</td>
<td>Als niet aan de criteria voor 5,5-7,5 is voldaan</td>
<td>De student gebruikt wetenschappelijke literatuur die ingaat op recente ontwikkelingen en geeft dit in scriptie voldoende weer.</td>
<td>+ De student laat zien literatuur op een hoger niveau te integreren in de scriptie</td>
<td></td>
</tr>
</tbody>
</table>

Toelichting

| Eindcijfer | 1+2+3+4+5+6/6 = |

Naam en handtekening Examinator/ docentbegeleider *: ……………………………………………………………………………………………………………………………………………………

NB* = weghalen wat niet van toepassing is.

B= Beroepscompetenties Opleiding Voeding & Diëtetiek:
1. Advisering: Analyseren van problemen vragen en behoeften op het gebied van voeding, doelen stellen gebaseerd op wetenschappelijke inzichten en afstemming met stakeholders en daarover adviseren, rekening houdend met relevante randvoorwaarden.
2. Begeleiding: Begeleiden van de uitvoering van adviezen gericht op problemen, vragen of behoeften op het gebied van voeding.
3. Rapportage: Registreren, evalueren en rapporteren van resultaten en procedures of richtlijnen ontwikkelen ter optimalisering van effecten van voeding
5. Management: Coördineren en aansturen van bedrijfsprocessen, gebaseerd op de strategie van de onderneming / organisatie / afdeling. Vaststellen strategie, beleid en planning van een organisatie.
6. Onderzoek: Opzetten en uitvoeren van voeding gerelateerd praktijkericht onderzoek, verwerken van gegevens en rapporteren van resultaten.

H= hbo-standaard
I  Een gedegen theoretische basis
II  Het onderzoekend vermogen
III  Professioneel vakmanschap
IV  Beroepsethiek en maatschappelijke oriëntatie